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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Douglas H. ROBINSON	)	
	)	
Serial No. 08/719,367	)	Examiner: J. Williams
	)	
Filed: September 25, 1996	)	Group Art Unit: 1815
	)	
For: METHODS FOR THE PRODUCTION OF BACTERIA CONTAINING EUKARYOTIC GENES	)	

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TECH CENTER 1600/2900

DECLARATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Douglas H. Robinson, M.D., declare as follows:

1. I am the same Douglas H. Robinson identified as the inventor of this application.

2. In broad terms, the present invention flows from my discovery that virally-infected eukaryotic cells can be cultured under certain anaerobic/partially-aerobic conditions so as to result in the production of bacteria that contain genetic material from the virus and/or the eukaryotic cell. This process involves the use of sterile culture medium and aseptic culture conditions, thus ensuring that the resulting bacteria did not arise from

contamination. I have referred to this process as the de novo speciation of bacteria from eukaryotic cells.

3. In the parent application, the P.T.O. questioned whether my reported results were due to contamination, and stated that "there is no clear correlation between the instant method of culturing and the production of new strains of bacteria."

4. In order to more fully explore my invention and in an attempt to have an independent entity corroborate my findings, I engaged Microbiological Associates, Inc., of Rockville, Maryland, to attempt to reproduce my process. Microbiological Associates ("MA") is a well-known and industry-recognized leader in the fields of testing biological materials, process validation and quality assurance. A true and exact copy of the entire report prepared by MA is attached hereto.

5. The work undertaken by MA was intended to reproduce the process disclosed in the above-identified patent application. Specifically, MA was able to reproduce the claimed process; bacteria were isolated from an RT-HCMV endothelial cell culture that was subjected to alternating anaerobic/aerobic conditions. A variety of sterility and mycoplasma tests were performed, and the results of those tests, too, are included in the attached report. The report is signed by Anton F. Steuer, Ph.D., an employee of MA and

the Study Director. Moreover, the report is accompanied by a Quality Assurance Statement that shows compliance with:

- U.S. F.D.A. Good Laboratory Practice Regulations ("GLP's") (21 CFR § 58);
- U.S. E.P.A. GLP's (40 CFR §§ 160 and 792);
- United Kingdom GLP Compliance Programme;
- the Japan GLP Standard; and
- the OECD Principles of Good Laboratory Practice.

6. The "Conclusion" of the report is unequivocal:

> Two runs involving the periodic reintroduction of an aerobic atmosphere during an anaerobic eukaryotic cell culture phase resulted in the isolation of bacteria, specifically *Bacillus licheniformis*. Four different colony morphologies were observed. Two runs in which an aerobic atmosphere was not periodically reintroduced during an anaerobic eukaryotic cell culture phase resulted in the isolation of no bacteria. All eukaryotic cell controls and media controls were negative. The isolation of bacteria from eukaryotic cells subjected to alternating anaerobic/aerobic cell culture conditions provides supporting evidence for the hypothesis of *de novo* evolution of bacteria from eukaryotic cells. On the other hand, the possibility of environmental contamination as the source of the bacterial isolates cannot be absolutely eliminated. Environmental contamination is unlikely due to the cGMP compliance procedures and practices employed in the performance of the sterility assays, which includes a stringent environmental and personnel monitoring program. Also, no tube, plate, or bottle inoculated with eukaryotic cell control samples or media control samples showed any microbial outgrowth. These negative results for all the numerous control samples tested minimized significantly the possibility of environmental contamination.

7. In view of the accompanying report (corroborating evidence provided by a well-respected, independent laboratory), I believe that all of the P.T.O.'s concerns regarding the ability of my process to work as described, and specifically to provide bacteria upon culturing eukaryotic cells as described, have been overcome. I respectfully request favorable treatment of my patent application directed to this invention.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and of any patent issued thereon.

Date: 2/25/97

Douglas H. Robinson, M.D.